Altered interactions between lipogenesis and fatty acid oxidation in regenerating rat liver

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1. The concentrations of malonyl-CoA, citrate, ketone bodies and long-chain acylcarnitine were measured in freeze-clamped liver samples from fed or starved normal, partially hepatectomized or sham-operated rats. These parameters were used in conjunction with measurements of the concentration of plasma non-esterified fatty acids and the rates of hepatic lipogenesis to obtain correlations between rates of fatty acid delivery to the liver, lipogenesis and fatty acid oxidation to ketone bodies and CO₂. 2. These correlations indicated that the development of fatty liver after partial hepatectomy is due to an increased partitioning of long-chain acyl-CoA towards acylglycerol synthesis and away from acylcarnitine formation. However, this did not appear to be due to an altered relationship between hepatic malonyl-CoA concentration and acylcarnitine formation. 3. For any concentration of long-chain acylcarnitine, the concentrations of both hepatic and blood ketone bodies were significantly lower in partially hepatectomized rats than in normal or sham-operated animals. This indicated that a lower proportion of the product of β -oxidation was used for ketone-body formation and more for citrate synthesis in the regenerating liver, especially during the first 24 h after resection. This inference was supported by the changes in hepatic citrate concentrations observed. 4. The high rates of lipogenesis that occurred in the liver remnant were accompanied by an altered relationship between lipogenic rate and hepatic malonyl-CoA concentration, such that much lower concentrations of malonyl-CoA were associated with any given rate of lipogenesis. 5. These adaptations are discussed in relation to the requirements by the remnant for high rates of energy formation through the tricarboxylic acid cycle during the first 24 h after resection, and the possibility that cycling between fatty acid oxidation and synthesis may occur to a greater degree in regenerating liver.

INTRODUCTION

Starvation is characterized by high rates of ketone-body formation in the liver of normal rats. This is due both to increased delivery of non-esterified fatty acids (NEFA) to the liver and to the diversion of a greater proportion of them towards oxidation rather than to acylglycerol synthesis. The partitioning of cytosolic long-chain acyl-CoA between formation of acylglycerol and acylcarnitine is subject to several control mechanisms (see Sugden & Williamson, 1982; Zammit, 1984), including changes in the activities of key enzymes of the two pathways and in the concentrations of the substrates of the initial reactions of the pathways. The inhibition of overt carnitine palmitoyltransferase (CPT I; EC 2.3.1.21) activity by malonyl-CoA provides a potent mechanism whereby low rates of fatty acid synthesis (e.g. during starvation) result, through lower malonyl-CoA concentrations, in de-inhibition of CPT I and increased rates of acylcarnitine formation. However, regulation of the rates of fatty acid esterification and oxidation can be exerted at sites other than the initial reactions of the two pathways (see Zammit, 1984). Thus both the total activity and the intracellular distribution of phosphatidate phosphohydrolase (E.C. 3.1.3.4) may change under conditions of increased esterification (Mangiapanne *et al.*, 1973). Similarly, the branch point represented by the partitioning of acetyl-CoA (formed from acylcarnitine)

between the synthesis of citrate and acetoacetyl-CoA is an additional site at which the rate of ketogenesis is determined (Krebs, 1966; Siess *et al.*, 1982).

Further work has demonstrated that, despite elevated plasma NEFA concentrations, the increases in blood and liver ketone-body concentrations, normally observed in response to starvation, are blunted during the first 2 days after partial hepatectomy (Schofield *et al.*, 1985a,b). Therefore the regenerating rat liver is a tissue in which the regulatory interactions mentioned above may be disturbed. The liver remnant shows increased incorporation of glycerol and plasma NEFA into triacylglycerol (Johnson & Alberts, 1960; Fex & Olivecrona, 1968; Olivecrona & Fex, 1970; Girard *et al.*, 1971). In addition, it has high rates of fatty acid synthesis (Gove & Hems, 1978). The latter does not occur in the intact liver under conditions of increased plasma concentrations of NEFA, owing to the inhibitory effects of long-chain acyl-CoA on acetyl-CoA carboxylase (EC 6.4.1.2) activity and the consequent lowering of hepatic malonyl-CoA concentration.

The present studies were conducted to elucidate the mechanisms through which these changes in metabolism (some of which are apparently paradoxical) are established in the liver remnant of partially hepatectomized rats. We measured the rates of lipogenesis and the concentrations of several intermediates in the liver of fed or starved normal, sham-operated or partially

Abbreviations used: NEFA, non-esterified fatty acids; CPT I, overt form of carnitine palmitoyltransferase.

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Table 1. Liver weights, blood concentrations of NEFA and ketone bodies and hepatic rates of lipogenesis in fed or starved control (unoperated), sham-operated or partially hepatectomized rats

Results are given as means $\pm$ S.E.M. for groups of four to six rats. Partially hepatectomized or sham-operated rats were killed at the post-operative day indicated, having been fed *ad libitum* or starved for the 24 h or 48 h immediately before sampling. Control rats were not subjected to any surgical procedure. Statistically significant effects of starvation are shown by: ^a*P* < 0.001; ^b*P* < 0.01; ^c*P* < 0.05. Statistically significant effects of partial hepatectomy compared with sham-operation are indicated by: ^d*P* < 0.001; ^e*P* < 0.01; ^f*P* < 0.05.

Parameter	Nutritional state	Control	Sham-operated		Partially hepatectomized	
			Day 1	Day 2	Day 1	Day 2
Liver wt. (g/100 g body wt.)	Fed <i>ad libitum</i>	4.46 $\pm$ 0.12	3.78 $\pm$ 0.20	3.98 $\pm$ 0.10	1.70 $\pm$ 0.13 ^d	2.47 $\pm$ 0.8 ^d
	24 h starved	3.19 $\pm$ 0.13 ^a	3.32 $\pm$ 0.09	3.18 $\pm$ 0.11 ^a	1.94 $\pm$ 0.11 ^d	2.30 $\pm$ 0.08 ^d
	48 h starved	2.75 $\pm$ 0.07 ^a	—	3.28 $\pm$ 0.08 ^a	—	2.73 $\pm$ 0.14 ^e
Plasma NEFA (mequiv./l)	Fed <i>ad libitum</i>	0.19 $\pm$ 0.01	0.23 $\pm$ 0.03	0.19 $\pm$ 0.06	0.45 $\pm$ 0.07 ^f	0.56 $\pm$ 0.06 ^e
	24 h starved	0.42 $\pm$ 0.05 ^a	0.43 $\pm$ 0.05 ^b	0.40 $\pm$ 0.03 ^a	0.95 $\pm$ 0.12 ^b	0.79 $\pm$ 0.01 ^d
	48 h starved	0.44 $\pm$ 0.04 ^a	—	0.74 $\pm$ 0.12 ^a	—	0.79 $\pm$ 0.12
Blood ketone bodies (mm)	Fed <i>ad libitum</i>	0.24 $\pm$ 0.05	0.29 $\pm$ 0.06	0.32 $\pm$ 0.05	0.28 $\pm$ 0.03	0.18 $\pm$ 0.06
	24 h starved	1.45 $\pm$ 0.27 ^a	2.10 $\pm$ 0.30 ^a	1.97 $\pm$ 0.22 ^a	0.43 $\pm$ 0.06 ^d	1.16 $\pm$ 0.23 ^{bf}
	48 h starved	2.67 $\pm$ 0.29 ^a	—	2.81 $\pm$ 0.30 ^a	—	1.40 $\pm$ 0.34 ^{cf}
Lipogenesis (μ g-atoms of ³ H/h per g wet wt.)	Fed <i>ad libitum</i>	18.6 $\pm$ 0.02	11.2 $\pm$ 1.0	14.8 $\pm$ 0.8	22.4 $\pm$ 1.8 ^e	19.4 $\pm$ 1.8 ^f
	24 h starved	9.4 $\pm$ 0.09 ^a	7.4 $\pm$ 1.2 ^c	6.8 $\pm$ 0.6 ^a	19.6 $\pm$ 3.6 ^e	13.0 $\pm$ 0.8 ^{cd}
	48 h starved	6.6 $\pm$ 0.02 ^a	—	6.8 $\pm$ 0.5 ^a	—	11.4 $\pm$ 0.5 ^{bd}

hepatectomized rats, as well as the plasma NEFA and blood ketone-body concentrations in these animals. The results suggest that in the regenerating liver an inherently increased diversion of acyl-CoA towards esterification is accompanied by increased utilization of acetyl-CoA for citrate synthesis. The possible relevance of this adaptation to the high rates of fatty acid synthesis in this tissue is discussed.

MATERIALS AND METHODS

Sources of materials were as described previously (Schofield *et al.*, 1985a,b).

Female albino Wistar rats (180–220 g) were subjected to a 12 h-light/12 h-dark cycle (light periods starting at 08:30 h) and were either fed *ad libitum* or starved for 24 h or 48 h in grid-bottomed cages before being killed. Partial hepatectomy (comprising laparotomy and removal of two-thirds of the liver) or sham operation (laparotomy) was performed under diethyl ether anaesthesia as described previously (Schofield *et al.*, 1985a). Rats were killed at 24 h or 48 h after the operation. Control rats were not subjected to either anaesthesia or surgery.

Experiments were started between 08:30 and 09:30 h. Rates of lipogenesis were estimated as ³H incorporation from ³H₂O into tissue saponifiable fatty acid as described by Stansbie *et al.* (1976). Arterial samples were withdrawn for determination of specific radioactivities of plasma ³H₂O and concentrations of NEFA and ketone bodies (acetoacetate and 3-hydroxybutyrate). In a second series of experiments, portions of freeze-clamped liver were used for measurement of concentrations of the ketone bodies (Williamson *et al.*, 1962), malonyl-CoA (Beynen *et al.*, 1979), citrate (Dagley, 1974) and long-chain acylcarnitine as free carnitine after alkaline hydrolysis (Cederblad & Lindsted, 1972).

Statistical significance of differences was assessed by

Student's unpaired *t* test. Results are given as means $\pm$ S.E.M. for four to six rats in each group.

RESULTS AND DISCUSSION

The conditions studied, namely feeding *ad libitum* and 24 h or 48 h starvation of normal, sham-operated or partially hepatectomized rats, resulted in a wide range of plasma NEFA concentrations in all three experimental groups. Consequently, we were able to correlate the changes in hepatic metabolic parameters with changes in the rate of delivery of fatty acids to the liver. The latter would, in fact, be higher than suggested solely by plasma NEFA concentrations in partially hepatectomized rats, because of the increased blood supply to the liver remnant (Brauer, 1963).

We confirmed the observation by Gove & Hems (1978) that the liver remnant shows high rates of lipogenesis (Table 1). This is thought to be mostly due to increased fatty acid synthesis, since, although the method used also includes ³H incorporated into cholesterol, the degree of cholesterogenesis is relatively low ($\sim 5\%$), especially during the first 48 h after partial hepatectomy (Field *et al.*, 1985). An inverse relationship between plasma NEFA concentrations and hepatic lipogenesis was observed for all conditions tested. However, whereas this relationship was identical for normal and sham-operated rats, much higher plasma concentrations of NEFA were required to produce a smaller degree of inhibition of lipogenesis in the liver of partially hepatectomized rats (Fig. 1). This was particularly apparent for rats starved during the first 24 h post-operatively. This relative insensitivity of the rate of lipogenesis to inhibition by fatty acid metabolism by the remnant was associated with a much altered relationship between lipogenic rate and hepatic malonyl-CoA concentration (Fig. 2). Although in all three groups of animals the rate of fatty acid synthesis was proportional to malonyl-CoA

Fig. 1. Relationship between plasma NEFA concentrations and rates of hepatic lipogenesis *in vivo*

The mean values are abstracted from Table 1 and are plotted for normal (●, ○, ○), sham-operated (2 days, ▲, △, △; 1 day, ■, ▨) and partially hepatectomized (2 days, ◆, ◇, ◇; 1 day, ▽, ▽) rats. Filled symbols, fed rats; semi-filled symbols, starved for 24 h; empty symbols, starved for 48 h. See legend to Table 1 for details.

concentration (cf. Guynn *et al.*, 1972), the lipogenic rate was several-fold more sensitive to malonyl-CoA concentration in the liver of partially hepatectomized rats, such that a plot of lipogenic rate against [malonyl-CoA] was markedly steeper. If the affinity of fatty acid synthase for malonyl-CoA is not altered by partial hepatectomy, this increased sensitivity of lipogenic rate to changes in malonyl-CoA concentration must reflect a higher maximal activity of fatty acid synthase. The maximal activity of acetyl-CoA carboxylase is reported not to be affected by partial hepatectomy (Gove & Hems, 1978), although the expressed activity of this enzyme could be altered by covalent modification or allosteric effects. Irrespective of the precise mechanism involved, the present data indicate that a higher effective fatty acid synthase/acetyl-CoA carboxylase activity ratio exists in the regenerating liver.

As expected from previous reports (Blackshear *et al.*, 1974; Schofield, 1985a,b) of the milder ketonaemia that accompanies starvation in partially hepatectomized rats (compared with normal), the relationship between plasma NEFA and blood ketone-body concentrations was altered (Fig. 3) (very similar results were obtained when hepatic ketone-body concentrations were used; results not shown). Neither hepatic nor blood concentrations of ketone bodies are necessarily accurate indices of the rate of ketogenesis. [However, it is unlikely that the low hepatic and blood ketone-body concentrations were due solely to decreased liver mass. Preliminary experi-

Fig. 2. Dependence of the rate of lipogenesis on hepatic malonyl-CoA concentrations in the liver of normal, sham-operated and partially hepatectomized rats

Mean values are abstracted from Tables 1 and 2. See legend to Fig. 1 for details of notation.

ments indicated that ketone-body concentrations were always lower in animals starved for 48 h immediately post-operatively compared with those starved 2 days later, despite a near-constant liver mass (results not shown)]. Nevertheless, these parameters have proved useful in previous studies (see, e.g., Zammit, 1981) in deducing interrelationships between hepatic metabolic processes. The present observations suggest that, for any given rate of hepatic fatty acid utilization, a smaller proportion was converted into ketone bodies in partially hepatectomized rats. This appeared to be due to two mechanisms. (i) Decreased acylcarnitine synthesis. The dependence of hepatic acylcarnitine concentrations on plasma NEFA concentrations was altered after partial hepatectomy such that, for an equivalent rate of delivery of fatty acids to the liver remnant, a lower concentration of long-chain acylcarnitines resulted (Fig. 4). This observation is compatible with the suggestion that acylglycerol synthesis is activated in the regenerating liver (Mangiapanne *et al.*, 1973). However, it is noteworthy that this switch in fatty acid metabolism towards esterification does not appear to result from any inordinate inhibition of CPT I activity by malonyl-CoA (Fig. 5). (ii) Increased citrate synthesis. A comparison of the dependence of blood ketone-body concentrations on acylcarnitine concentrations in the livers of all three groups of animals studied indicated that the relationship

Fig. 3. Relationship between plasma NEFA and blood ketone-body concentrations in normal, sham-operated and partially hepatectomized rats

See legend to Fig. 1 for details of notation.

Fig. 4. Relationship between hepatic acylcarnitine concentrations and plasma NEFA concentrations in normal, sham-operated and partially hepatectomized rats

See Tables 1 and 2 and legend to Fig. 1 for details.

between the two parameters was altered after partial hepatectomy: for equivalent concentrations of acylcarnitine, the circulating ketone-body concentrations were lower (Fig. 6). The same relationship was observed between plasma [NEFA] and hepatic ketone-body

Fig. 5. Relationship between hepatic malonyl-CoA and acylcarnitine concentrations in liver of normal, sham-operated and partially hepatectomized rats

See legends to Tables and Fig. 1 for details.

Fig. 6. Relationship between hepatic acylcarnitine concentrations and blood ketone-body concentrations

See legend to Fig. 1 for details of notations.

Table 2. Liver ketone bodies, citrate, malonyl-CoA and long-chain acylcarnitine concentrations in fed or starved control (unoperated), sham-operated and partially hepatectomized rats

For details see the legend to Table 1. Results are given as means $\pm$ S.E.M. for groups of four to six rats. Statistically significant effects of starvation are shown by: ^a $P < 0.001$; ^b $P < 0.01$; ^c $P < 0.05$. Statistically significant effects of partial hepatectomy compared with sham operation are indicated by: ^d $P < 0.001$; ^e $P < 0.01$; ^f $P < 0.05$.

Nutritional state		Sham-operated			Partially hepatectomized	
		Control	Day 1	Day 2	Day 1	Day 2
3-Hydroxybutyrate (μ mol/g wet wt.)	Fed <i>ad libitum</i>	0.168 $\pm$ 0.021	0.227 $\pm$ 0.070	0.221 $\pm$ 0.066	0.244 $\pm$ 0.052	0.163 $\pm$ 0.011
	24 h starved	0.813 $\pm$ 0.140 ^b	1.130 $\pm$ 0.199 ^a	0.950 $\pm$ 0.083 ^a	0.344 $\pm$ 0.036 ^d	0.686 $\pm$ 0.107 ^b
	48 h starved	1.184 $\pm$ 0.144 ^a	—	1.081 $\pm$ 0.092 ^a	—	0.553 $\pm$ 0.033 ^{ad}
Acetoacetate (μ mol/g wet wt.)	Fed <i>ad libitum</i>	0.152 $\pm$ 0.010 ¹⁷	0.324 $\pm$ 0.088	0.290 $\pm$ 0.016	0.299 $\pm$ 0.033	0.216 $\pm$ 0.030
	24 h starved	0.220 $\pm$ 0.005 ^b	0.363 $\pm$ 0.035	0.388 $\pm$ 0.029 ^c	0.289 $\pm$ 0.004	0.250 $\pm$ 0.030 ^f
	48 h starved	0.405 $\pm$ 0.056 ^a	—	0.216 $\pm$ 0.016	—	0.144 $\pm$ 0.013 ^f
Citrate (μ mol/g wet wt.)	Fed <i>ad libitum</i>	0.235 $\pm$ 0.051	0.156 $\pm$ 0.027	0.271 $\pm$ 0.009	0.213 $\pm$ 0.022	0.149 $\pm$ 0.030 ^f
	24 h starved	0.189 $\pm$ 0.015	0.156 $\pm$ 0.017	0.199 $\pm$ 0.014 ^b	0.357 $\pm$ 0.032 ^{bd}	0.185 $\pm$ 0.028
	48 h starved	0.154 $\pm$ 0.011	—	0.155 $\pm$ 0.011 ^a	—	0.092 $\pm$ 0.018 ^f
Malonyl-CoA (nmol/g wet wt.)	Fed <i>ad libitum</i>	1.43 $\pm$ 0.38	0.58 $\pm$ 0.15	0.48 $\pm$ 0.26	0.44 $\pm$ 0.06	0.37 $\pm$ 0.07
	24 h starved	0.36 $\pm$ 0.16 ^c	0.10 $\pm$ 0.03 ^c	0.13 $\pm$ 0.02	0.10 $\pm$ 0.04 ^b	0.07 $\pm$ 0.04 ^b
	48 h starved	0.29 $\pm$ 0.11 ^b	—	0.19 $\pm$ 0.02	—	0.07 $\pm$ 0.03 ^{bf}
Long-chain acylcarnitine (nmol/g wet wt.)	Fed <i>ad libitum</i>	18.8 $\pm$ 1.9	21.7 $\pm$ 2.6	15.0 $\pm$ 1.9	39.2 $\pm$ 8.9	28.8 $\pm$ 2.1 ^d
	24 h starved	51.4 $\pm$ 4.0 ^a	34.8 $\pm$ 4.1 ^c	43.4 $\pm$ 5.0 ^a	59.4 $\pm$ 8.8 ^f	66.8 $\pm$ 13.9 ^c
	48 h starved	49.2 $\pm$ 3.5 ^a	—	71.9 $\pm$ 17.8 ^a	—	55.1 $\pm$ 6.7 ^b

content (results not shown). Since, as mentioned above, the rate of fatty acid oxidation is proportional to long-chain acylcarnitine concentration in hepatocytes (Christiansen *et al.*, 1976), these data suggest that a smaller proportion of acetyl-CoA derived from acylcarnitine oxidation is used for ketone-body synthesis after partial hepatectomy. This suggestion implies that acetyl-CoA is diverted towards citrate synthesis in the liver remnant, particularly in rats starved for 24 h post-operatively (Fig. 6). Circumstantial evidence for this interpretation was obtained from changes in the hepatic concentrations of citrate that occurred after starvation (Table 2). Thus, whereas in normal and sham-operated rats citrate concentrations tended to decrease after 24 h starvation, those in the liver of partially hepatectomized rats tended to increase. After 48 h starvation, citrate concentrations decreased in all three conditions, including partial hepatectomy, possibly reflecting the more similar dependence of ketone-body concentrations on acylcarnitine concentrations in the liver of these animals (Fig. 6).

If the suggestion that the liver remnant diverts more acetyl-CoA towards citrate is valid for animals starved for the first 24 h post-operatively, it would be expected that the mitochondrial concentration of oxaloacetate would be higher. This could result from a higher rate of pyruvate carboxylation in relation to the rate of oxaloacetate utilization for gluconeogenesis and/or to a less reduced state of the intramitochondrial NADH/NAD⁺ ratio (Wieland, 1968; see also Zammit, 1983). The mean [3-hydroxybutyrate]/[acetoacetate] ratio was markedly lower in liver of partially hepatectomized rats (1.19) than in those of normal (3.70) or sham-operated (3.11) rats starved for 24 h. This observation suggested that during the first 24 h after partial hepatectomy mitochondrial oxaloacetate concentrations may be high, thus favouring citrate synthesis. It is noteworthy that the

liver remnants of these animals also contained the highest concentrations of citrate observed (Table 2).

General discussion

Previous studies had indicated that partially hepatectomized rats do not develop marked ketonaemia after starvation. In the present study we have obtained evidence for the identification of the intrahepatic mechanisms responsible. Two main adaptations appear to be involved. (i) Increased diversion of long-chain acyl-CoA towards acylglycerol formation (this is important during the entire 48 h period post-operatively). It is essential to stress that, because the blood flow to the remnant is markedly increased (per unit weight) compared with normal liver (Brauer, 1963), the changes in the relationships between rate of NEFA delivery to the liver and hepatic metabolic parameters would be even more exaggerated than suggested in Figs. 1, 3 and 4. (ii) Increased synthesis of citrate owing to higher oxaloacetate concentrations (this appears to be important primarily when animals are starved for the first 24 h after partial hepatectomy). Citrate can be used either for oxidation through the tricarboxylic cycle or for provision of cytosolic acetyl-CoA. It is plausible that tricarboxylic acid cycle activity is increased in the liver remnant, considering the considerable energy demands that regeneration imposes, especially during the first 24 h when cell growth is occurring before division. The increased rate of lipogenesis in the remnant may also be related to increased citrate formation both through the effects of the tricarboxylic acid on acetyl-CoA carboxylase and because of increased availability of cytosolic acetyl-CoA. It is possible that in the regenerating rat liver some cycling of carbon occurs between fatty acid oxidation and synthesis. This may contribute towards the high rates of fatty acid synthesis observed in the tissue (Gove & Hems, 1978; the present paper). In the liver of

normal animals, such cycling is minimized through the direct dependence of the rate of fatty acid synthesis on malonyl-CoA concentration on the one hand and the inverse relationship between malonyl-CoA concentration and CPT I activity on the other. However, the observed altered relationship between the rate of fatty acid synthesis and malonyl-CoA concentration in the regenerating liver may result in substantial simultaneous rates of both pathways by allowing high rates of lipogenesis at concentrations of malonyl-CoA that are not sufficiently inhibitory to CPT I.

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REFERENCES

- Beynen, A. C., Vaartjes, W. J. & Geelen, M. J. H. (1979) *Diabetes* **28**, 828–835
- Blackshear, P. J., Holloway, P. A. H. & Alberti, K. G. M. M. (1974) *Biochem. J.* **142**, 279–286
- Brauer, R. W. (1963) *Physiol. Rev.* **43**, 115–213
- Cederblad, G. & Lindsted, S. (1972) *Clin. Chim. Acta* **37**, 235–243
- Christiansen, R.-Z., Borrebaeck, B. & Bremer, J. (1976) *FEBS Lett.* **62**, 313–317
- Dagley, S. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1562–1565, Academic Press, New York
- Fex, G. & Olivecrona, T. (1968) *Biochim. Biophys. Acta* **152**, 237–243
- Field, F. J., Mathews, S. N. & La Brecque, D. R. (1985) *Am. J. Physiol.* **249**, G679–G684
- Girard, A., Roheim, P. S. & Eden, H. A. (1971) *Biochim. Biophys. Acta* **248**, 105–113
- Gove, C. D. & Hems, D. A. (1978) *Biochem. J.* **170**, 1–8
- Guynn, R. W., Veloso, D. & Veech, R. L. (1972) *J. Biol. Chem.* **247**, 7325–7331
- Johnson, R. M. & Alberts, S. (1960) *J. Biol. Chem.* **235**, 1299–1302
- Krebs, H. A. (1966) *Adv. Enzyme Regul.* **4**, 339–353
- Mangiapan, E. H., Lloyd-Davies, K. A. & Brindley, D. N. (1973) *Biochem. J.* **134**, 103–112
- Olivecrona, T. & Fex, G. (1970) *Biochim. Biophys. Acta* **202**, 259–268
- Schofield, P. S., French, T. J., Goode, A. W. & Sugden, M. C. (1985a) *FEBS Lett.* **184**, 214–220
- Schofield, P. S., McLees, D. J., Myles, D. D. & Sugden, M. C. (1985b) *Biochem. J.* **231**, 225–228
- Siess, E. A., Kientsch-Engel, R. I. & Wieland, O. H. (1982) *Eur. J. Biochem.* **121**, 493–499
- Stansbie, D., Brownley, R. W., Crettaz, M. & Denton, R. M. (1976) *Biochem. J.* **160**, 413–416
- Sugden, M. C. & Williamson, D. H. (1982) in *Metabolic Compartmentation* (Sies, H., ed.), pp. 287–316, Academic Press, London
- Wieland, O. H. (1968) *Adv. Metab. Disord.* **3**, 1–47
- Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96
- Zammit, V. A. (1981) *Biochem. J.* **198**, 75–83
- Zammit, V. A. (1983) *Proc. Nutr. Soc.* **42**, 289–302
- Zammit, V. A. (1984) *Prog. Lipid Res.* **23**, 39–67

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